NOV 17 1917

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXIII NOVEMBER, 1917 NO. 5

CONTENTS

		PAGE
WHITNEY, D. D.	*The Production of Functional and Rudimentary Spermatozoa in Rotifers*	305
PORTER, LELIA T.	*The Spermatocytic Divisions of Leptocoris hæmatoloma*	316
CHARLTON, HARRY H.	*The Fate of the Unfertilized Egg in the White Mouse*	321
WEBER, ROXIE A.	*Observations on the Structure of Double Monsters in the Earthworm*	339
WOODWARD, ALVALYN E., AND HAGUE, FLORENCE S.	*Iodine as a Parthenogenetic Agent*	355

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY
& SON
28 Essex Street, Strand
London, W. C.

AGENT FOR GERMANY
R. FRIEDLÄNDER
& SOHN
Berlin N. W.
Carlstrasse, 11

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

Jacques Loeb—*The Rockefeller Institute for Medical Research*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXIII. *November, 1917.* *No. 5.*

BIOLOGICAL BULLETIN

THE PRODUCTION OF FUNCTIONAL AND RUDIMENTARY SPERMATOZOA IN ROTIFERS.[1]

D. D. WHITNEY.

Marked dimorphism in the spermatozoa of a few animals has been briefly recorded by several observers, but no detailed study has been made of such forms to determine how the two kinds of spermatozoa are formed nor has there been offered an explanation as to their probable functions. While looking at the plates in "The Rotifera," by Hudson and Goss, I noticed sketches of the two dimorphic spermatozoa of the marine rotifer, *Brachionus mulleri*. As this rotifer occurs in countless numbers in a salt-water lake near Lincoln, Neb., an excellent opportunity was offered to reëxamine it in an attempt to find an explanation for the production of the two kinds of spermatozoa. After reëxamining the spermatozoa of this rotifer and confirming the observations of Hudson and Goss the spermatozoa of eight other species of rotifers were examined and were found also to be composed of two kinds. Thus indicating that this phenomenon of dimorphism of spermatozoa may be general for all the species of the whole group of rotifers which produce male individuals.

The technique of examining the spermatozoa is very simple. Mature free swimming males were placed on a slide in a drop of the culture water and covered with a cover glass. Then by pressure the males were forced to eject the contents of the single testis into the water. The two kinds of spermatozoa could be readily seen even with the low powers of the microscope and were as easily counted. Early stages in the development of the spermatozoa as far back as the early spermatids were studied by crushing the eggs containing the unhatched immature males. All stages of development from the earliest spermatid up through

[1] Studies from the Zoölogical Laboratory, The University of Nebraska, No. 117.

to a mature spermatozoön were easily seen, especially in the species of rotifers whose males form only a few spermatozoa.

Brachionus mulleri, the salt-water rotifer, was the first species examined and because of the relatively small number of spermatozoa produced by the males it proved to be the most favorable form in which to see the early stages in the development of the spermatozoa as well as for making accurate counts. The motile spermatozoa were extraordinarily large and measured about one third the total length of the male individual that produced them. Probably on account of the large size of the spermatozoa a male is physically unable to form very many. These motile spermatozoa were long and somewhat slender with a pointed anterior end. The posterior two-thirds of the spermatozoön is very motile and has a prominent, undulating, median, and dorsal membrane which aids greatly in the locomotion. The sketch of a mature spermatozoön in Fig. 1, *E*, is a dorsal view and does not show adequately this membrane. These spermatozoa swim freely about in the water for a short time after they are forced out from the body of the male.

The spindle-shaped spermatozoa are incapable of any locomotion. They are uniform in shape and never change their form. They are very much smaller than the motile spermatozoa. Fig. 2, *D*, shows several of these. As the tissues of the males are very transparent both kinds of these spermatozoa may be readily seen inside the testis. The spindle-shaped spermatozoa are usually found collected together near the posterior end of the testis, although sometimes only a few are thus collected together while the remainder are scattered throughout the testis among the motile spermatozoa.

When the immature males are crushed the spermatids still united in masses separate readily from the general debris. Various stages in the development of the spermatids and spermatozoa may be seen in accordance with the stage of development of the male. All spermatids or immature spermatozoa in each male are generally found in the same stage of development.

The relative number of the two kinds of spermatozoa varied widely when counts were made from the mature free swimming males taken from a general culture of rotifers. It is very prob-

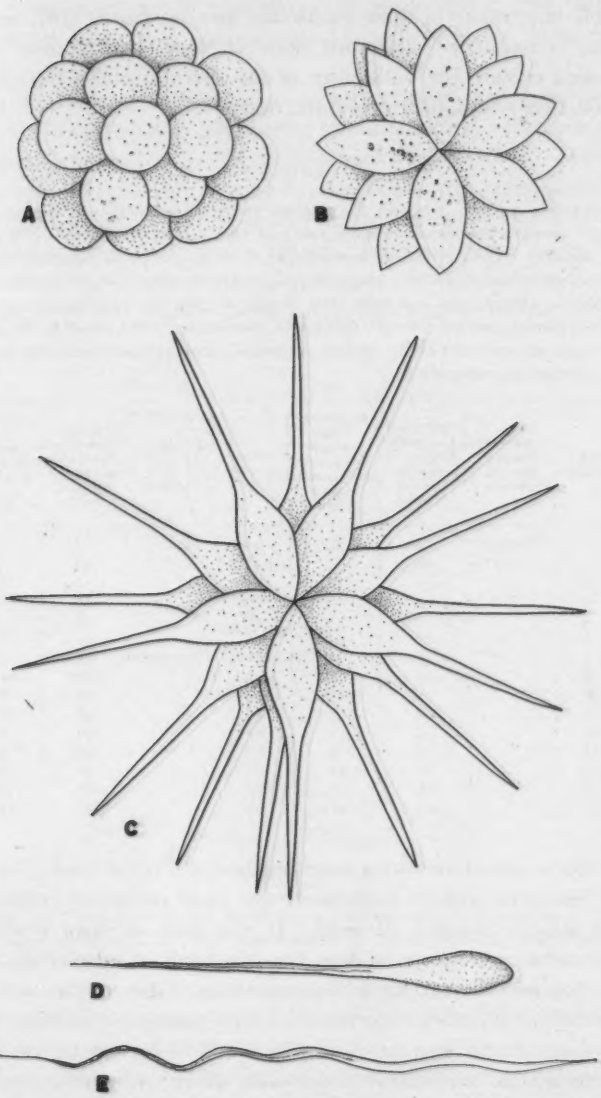


FIG. 1. Formation of the functional spermatozoa in *Brachionus mulleri*. *A*, young spermatids; *B*, older spermatids; *C*, young immature spermatozoa; *D*, older immature spermatozoön; *E*, mature, motile, and functional spermatozoön.

able that many of these males had already paired with females and consequently had shed some of their spermatozoa. This would explain the variability of the spermatozoa counts in the first twenty-one males recorded in Table I.

TABLE I.

Brachionus mulleri. Showing the relative proportional number of the two kinds of spermatozoa found in each testis of thirty male individuals. The rudimentary spermatozoa were never found as numerous as the functional spermatozoa in any testis of a young male. In the immature males in which none of the spermatozoa had been fully formed or shed the larger spermatids that ultimately develop into the functional spermatozoa were found to be exactly twice as numerous as the smaller spermatids that ultimately develop into the rudimentary spermatozoa.

Serial Number.	State of Development of Males.	Number of Functional Spermatids or Spermatozoa in Testis.	Number of Rudimentary Spermatids or Spermatozoa in Testis.	Serial Number.	State of Development of Males.	Number of Functional Spermatids or Spermatozoa in Testis.	Number of Rudimentary Spermatids or Spermatozoa in Testis.
1	Mature	50	26	16	Mature	5	0
2	"	48	—	17	"	24	8
3	"	—	12	18	"	9	10
4	"	—	0	19	"	28	10
5	"	6	2	20	"	18	6
6	"	—	15	21	"	60	25
7	"	2	1	22	Immature	38	18
8	"	20	11	23	"	70–80	38–40
9	"	—	18	24	"	40	18–20
10	"	22	13	25	"	40	18–20
11	"	4	0	26	"	60	32
12	"	15	5	27	"	64	32
13	"	56	35	28	"	40	20
14	"	28	11	29	"	56	28
15	"	40	22	30	"	48	24

When immature males were crushed one could readily count all the spermatids of both kinds and could definitely determine the relative number of each. It was seen at once when all spermatids were counted that the two kinds of spermatids that develop into the two kinds of spermatozoa did not occur in equal numbers. The large spermatids which ultimately develop into the large motile spermatozoa were found to be exactly twice as numerous as the smaller spermatids which ultimately develop into the small spindle-shaped spermatozoa. This is clearly shown in the last few males recorded in Table I. Figs. 1 and 2

show a series of the various stages of development from early spermatids to mature spermatozoa of both kinds of these dimorphic spermatozoa.

Mature males of *Hydatina senta* were crushed and examined and were found also to possess the two kinds of spermatozoa as markedly dimorphic as those in *Brachionus mulleri*. Many more spermatozoa are formed in the testis and consequently the counts could not be as readily or as accurately made as in *Brachionus mulleri*. Counts enough, however, were made to show that the large motile spermatozoa were about twice as numerous as the smaller immotile spindle-shaped spermatozoa.

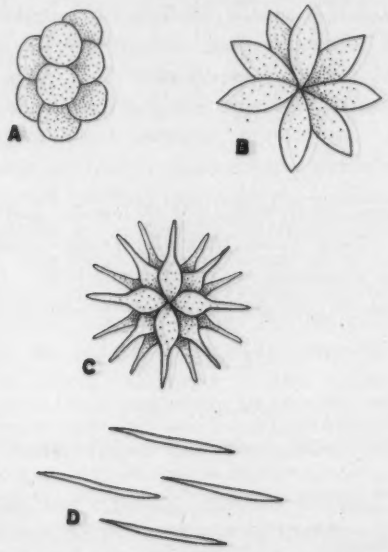


FIG. 2. Formation of the rudimentary spermatozoa in *Brachionus mulleri*. *A*, young spermatids; *B*, older spermatids; *C*, young immature spermatozoa; *D*, mature, immotile, and rudimentary spermatozoa.

The counts are shown in Table II. and drawings of the two kinds of spermatozoa are shown in Fig. 5.

The spermatozoa were examined next of the large viviparous rotifer, *Asplanchna amphora*, and although no very accurate counts could be made the two kinds of spermatozoa were con-

spicuously present. The highest count for the motile spermatozoa was about 200, while the highest count of the immotile spindle-shaped spermatozoa was about 100. These counts were made from males which were isolated at birth before they

TABLE II.

Hydatina senta. Showing the relative proportional number of the mature dimorphic spermatozoa found in each testis. In general the large motile spermatozoa were twice as numerous as the small spindle-shaped spermatozoa.

Serial No.	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.	Serial No.	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.
1	Mature	60	18	8	Mature	—	50–60
2	"	—	24	9	"	50–60	25–30
3	"	72	26	10	"	0	5
4	"	160–200	—	11	"	20	18
5	"	130	44	12	"	186	—
6	"	—	40	13	"	90	52
7	"	—	30				

had had an opportunity to pair with females and to shed their spermatozoa. They show that the motile spermatozoa are about twice as numerous as the immotile kind. Drawings and counts of these two kinds of spermatozoa are shown in Fig. 3 and Table III.

TABLE III.

Asplanchna amphora. Showing the relative proportional number of the mature dimorphic spermatozoa found in each testis. In general the large motile spermatozoa were twice as numerous as the small spindle-shaped spermatozoa.

Serial Number	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.
1	Mature	200	50
2	"	200	60
3	"	—	104
4	"	160	75

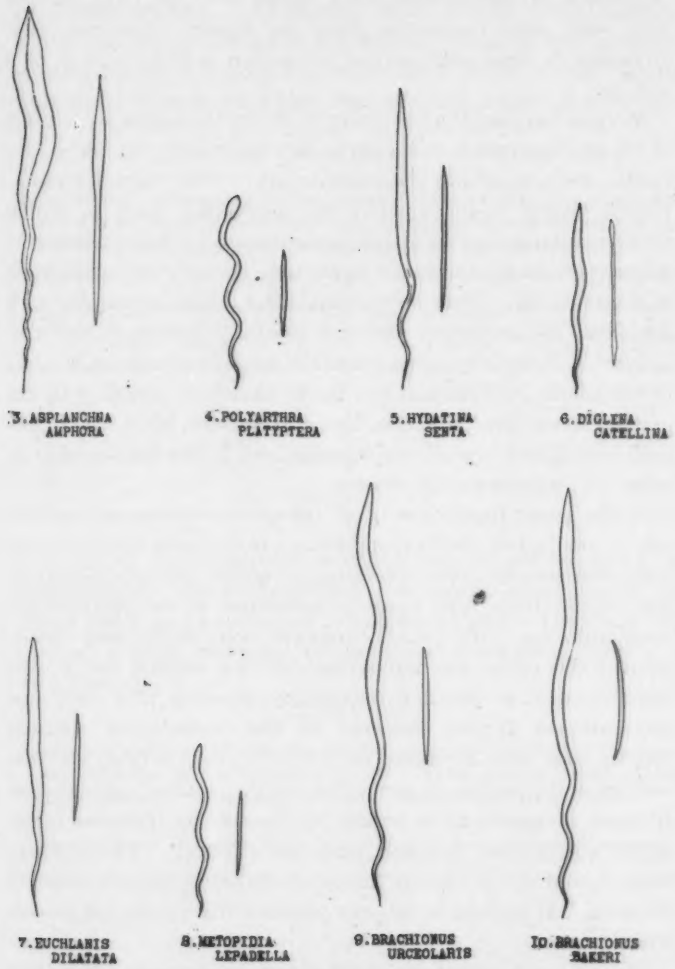
In addition to the spermatozoa of these three species of rotifers already described the spermatozoa of six other species of rotifers were carefully examined. These species were *Polyarthra platyptera*, *Diglena catellina*, *Euchlanis dilatata*, *Metopidia lepadella*, *Brachionus urceolaris* and *Brachionus bakeri*. In all cases the

two kinds of spermatozoa were found and the larger motile ones were more numerous than the smaller immotile ones. Drawings of these spermatozoa are shown in Figs. 4, 6, 7, 8, 9 and 10.

Morgan has found in the study of the spermatogenesis of some of the phylloxerans that the secondary spermatocytes are of two kinds—the normal and the rudimentary. The normal spermatocytes divide again to form the spermatids, each of which develops later into a functional spermatozoön. The rudimentary spermatocytes do not divide again and do not form spermatids or spermatozoa. They merely persist for a time as rudimentary secondary spermatocytes and then finally disintegrate and disappear. Morgan has also found the same phenomenon in some of the aphids, Stevens and von Baehr have also found it in the aphids, Meves has found it in *Vespa germanica*, Mark and Copeland have found it in *Vespa maculata*, and Lams has found it in an ant, *Campodinotus herculaneus*.

In the grand total count of all the spermatocytes and spermatids at the end of the final spermatocyte divisions in all of these forms enumerated in the preceding paragraph the normal spermatids should have been twice as numerous as the rudimentary spermatocytes. No counts, however, were made and consequently this point was not settled by this method but it was demonstrated in another manner by showing that only one spermatocyte division occurred in the rudimentary spermatocytes and two divisions occurred in the normal spermatocytes in the process of formation of the normal spermatozoa. In 1909 Morgan said in regard to the rotifer, *Hydatina senta*, which at the time had not been investigated: "Theoretically there should not be two spermatocyte divisions but only one true division. It remains to be seen whether this prediction proves true."

The rotifers have not only fulfilled but have exceeded Morgan's expectations! They not only produce normal spermatids and rudimentary secondary spermatocytes in the ratio of 2:1 as probably do these forms already mentioned, but they develop both of these into spermatozoa which are later shed by the males. Those of one kind are large, motile and probably functional while those of



FIGS. 3–10. Dimorphic spermatozoa of eight species of rotifers showing the larger, motile, and functional spermatozoa and the smaller, immotile, and rudimentary spermatozoa. In all of these species the drawings were made of spermatozoa that were taken from mature free-swimming males except in Fig. 4 which were made of spermatozoa of unhatched immature males. All were drawn to same scale as Figs. 1–2.

the other kind are small, immotile and probably rudimentary, and without function. The rudimentary spermatozoa must necessarily develop directly from the secondary spermatocytes without the occurrence of the second spermatocyte division in order to form the ratio of two normal spermatozoa to one rudimentary spermatozoön. If they developed from rudimentary spermatids which were formed by the division of the secondary spermatocytes the ratio would be one functional spermatozoön to one rudimentary spermatozoön. This interpretation of the observed ratios would therefore place the rotifers in the same category as the other animals already mentioned as far as the formation of normal spermatids and of rudimentary secondary spermatocytes are concerned. They would, however, have this point of difference, viz.: that the degree of degeneration of these rudimentary secondary spermatocytes has not progressed as far as to render such spermatocytes incapable of developing directly into degenerate spermatids and spermatozoa of a certain kind, as is the case of the other forms studied.

In the mature parthenogenetic egg that develops into the male individual there is the reduced number of chromosomes. This contains one half as many chromosomes as are found in the parthenogenetic egg that develops into the female. This fact was made out in *Hydatina senta* but the exact number of chromosomes occurring in each kind of egg has never been determined. If the male in the beginning has already the reduced number of chromosomes no further reduction of chromosomes is necessary in the formation of the spermatozoa. The race, however, may have retained the former ancestral process of performing the first spermatocyte division, regardless of the results, in the formation of the secondary spermatocytes but as there is no general reduction of chromosomes to be made at this stage presumably none occurs. One half of the secondary spermatocytes very likely contain fewer or perhaps no chromosomes at all and consequently are very degenerate and rudimentary and without a second division develop directly into degenerate spermatids and spermatozoa.

The male parthenogenetic eggs are the ones that are fertilized as has been determined by Maupas, Lauterborn, Whitney, and

Shull and after fertilization always develop into females but never into males. Thus the fertilization causes a change of the sex in the resulting individual from such eggs. All of the functional spermatozoa must therefore be identical in their influence in producing females from eggs that if unfertilized would otherwise have produced males.

SUMMARY.

1. Dimorphic spermatozoa are produced and shed by the rotifers, *Brachionus mulleri*, *Asplanchna amphora*, *Polyarthra platyptera*, *Hydatina senta*, *Diglena catellina*, *Euchlanis dilatata*, *Metopidia lepadella*, *Brachionus urceolaris* and *Brachionus bakeri*.

2. One class of the spermatozoa is composed of large, vermiform, and motile spermatozoa and the other class is composed of small, spindle-shaped and immotile spermatozoa.

3. The number of motile spermatozoa produced is exactly twice the number of immotile spermatozoa produced.

4. The motile spermatozoa probably develop from the cells formed by the second division of the normal spermatocytes but the immotile spermatozoa probably develop directly from the rudimentary cells formed by the first division of the spermatocytes.

5. The motile spermatozoa are probably functional but the immotile spermatozoa are probably rudimentary and functionless.

6. The functional spermatozoa are all identical in their power of determining the sex of the individual that develops from a fertilized egg. After a functional spermatozoön has fertilized a parthenogenetic male egg the egg eventually develops into a female individual instead of developing into a male individual as it otherwise would have done if it had not been fertilized.

ZOÖLOGICAL LABORATORIES,
THE UNIVERSITY OF NEBRASKA,
LINCOLN, NEBRASKA,
June 22, 1917.

LITERATURE CITED.

Von Baehr, W. B.

'08 Ueber die Bildung der Sexualzellen bei Aphididæ. Zoöl. Anz., Vol. 33.

Gruvel, A.

'05 Journ. Roy. Micro. Soc., p. 34.

November 3.—In some recent and more careful studies on the spermatogenesis of *Brachionus mulleri* it has been determined that the bodies which have been designated as normal spermatozoa are only the detached tails of these spermatozoa and are not the complete cell. When the males are compressed under a cover glass the tails become detached from the heads of the spermatozoa while inside the body of the males and then are immediately extruded and remain in an active state for a considerable length of time. This fact was not observed by Hudson and Goss as is shown by their plate 27 nor by myself until very recently.

As these motile tails resemble very closely the motile spermatozoa of the other species of rotifers described in this paper it is very probable that all the motile and supposedly complete spermatozoa observed were also only tails. Whether the bodies that were considered rudimentary spermatozoa are only tails of such spermatozoa or are complete spermatozoa has not been definitely decided.

These new detailed facts do not, in the least manner, invalidate the main issue of the paper in regard to the two classes of spermatozoa and their ratio. Additional observations and data derived from these recent studies will be published shortly.

THE AUTHOR.

Hudson and Goss.

'86 The Rotifera. Vols. 1–2. Longmans, Green, and Company.

Lams, H.

'08 Les Divisions des Spermatocytes chez la Fourmi. Arch. f. Zellfor., I.

Lauterborn.

'98 Ueber die cyclische Fortpflanzung limnetischer Rotatorien. Biol. Centrlbl., Vol. 18.

Lenssen.

'98 Contribution à l'etude du developpement et de la maturation des eufs chez Hydatina senta. La Cellule, Vol. 14.

Mark, E. L., and Copeland, U.

'07 Maturation Stages in the Spermatogenesis of Vespa maculata. Proc. Am. Acad. Arts and Sci., Vol. 43.

Maupas, M.

'90 Sur la fecondation de l'Hydatina senta. C. R. Acad. Sci., Paris, Vol. III.

Meves, Fr., und J. Duesberg.

'08 Die Spermatozytenteilungen bei der Hornisse. Arch. f. mikr. Anat., Vol. 71.

Morgan, T. H.

'08 The Production of Two Kinds of Spermatozoa in Phylloxerans—Functional "Female Producing" and Rudimentary Spermatozoa. Proc. Soc. Exp. Biol. and Med., Vol. 5.

'09 A Biological and Cytological Study of Sex Determination in Phylloxerans and Aphids. Jour. Exper. Zoöl., Vol. 7.

O'Donohoe, T. A.

'11 Dimorphism in the Spermatozoa of the Flea and Blow-Fly. Journ. Quek. Micro. Club, Vol. II.

Shull, A. F.

'10 Studies in the Life Cycle of Hydatina senta. Journ. Exper. Zoöl., Vol. 8.

Stevens, N. M.

'09 An Unpaired Chromosome in the Aphids. Journ. Exper. Zoöl., Vol. 6.

Whitney, D. D.

'07 Determination of Sex in Hydatina senta. Jour. Exper. Zoöl., Vol. 5.

'09 Observations on the Maturation Stages of the Parthenogenetic and Sexual Eggs of Hydatina senta. Jour. Exper. Zoöl., Vol. 6.

'13 An Explanation of the Non-Production of Fertilized Eggs by Adult Male-Producing Females in a Species of Asplanchna. BIOL. BULL., Vol. 25.

THE SPERMATOCYTIC DIVISIONS OF LEPTOCORIS HÆMATOLOMA.[1]

LELIA T. PORTER.

INTRODUCTION.

The spermatogenesis of *Leptocoris hæmatoloma* (family Coreidæ) very closely resembles that of *Anasa tristis*, as described by Paulmier. During the past few years it has been used extensively here in the course on cytology to demonstrate the behavior of the X-chromosome. As a matter of record, it seems worth while to give a very brief account of the spermatocytic divisions. Furthermore, the material is very fine for the study of tetrad formation, and it is hoped later to publish an account of this process.

The material on which these observations were made was collected in November and December, 1914, on the plant *Cardiospermum halicacabum*, the balloon vine which is very common in this vicinity. It may be of interest, however, to know that this is the only plant on which we have found this bug. The testes, which are large yellow pear-shaped bodies located at the base of the third pair of walking legs were dissected out in Ringer's solution and fixed immediately in Bouin's fluid. They were stained with iron hæmatoxylin and counterstained with eosin and light-green. Other fixing agents were used, but this proved to be the best.

The material is excellent for the study of spermatogenesis, as the chromosomes are not numerous, and vary greatly in size. It is possible to follow accurately the courses of the smallest, largest and accessory chromosomes throughout their entire history.

The spermatogonial complex of *Leptocoris hæmatoloma* consists of thirteen chromosomes (Figs. 1 and 2). These vary in size, but can be classified in three groups. The members of two pairs

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 138.

are quite large, three pairs are of medium size, while the sixth pair and the accessory chromosome are rather small. On account of their size and shape, one has little difficulty in recognizing the members of the various pairs.

After a series of successive spermatogonial divisions, the thirteen chromosomes pass through the characteristic synaptic and growth stages, giving rise to six tetrads and one dyad, typical of the primary spermatocyte. These tetrads vary greatly in size, the largest and smallest being easily recognized in Figs. 3 and 4. Three of the tetrads approximate each other very closely, and the remaining one is slightly smaller than the largest. The dyad representing the accessory chromosome lies close to the nuclear wall in Figs. 3 and 4, but I have no reason to believe that this is necessarily characteristic. Figs. 3 and 4 were selected because in each the entire number is seen, though scattered irregularly throughout the nucleus. At this stage the nuclear membrane is intact and the cell itself has increased enormously in size.

The tetrads are then drawn into the central plate and there is no question as to their final arrangement Fig. 5 shows the typical position of the various chromosomes in the central plate of the first spermatocyte. I have examined numbers of cells and at this stage have always found the six chromosomes arranged in a ring with the smallest in the center. The accessory chromosome always lies in the circle. Paulmier has found this to be the case in *Podisus*, *Lygæus* and *Chariesterus*, but Henking finds no such peculiar arrangement in *Pyrrochoris*.

First Spermatocytic Division.

The spindle of the primary spermatocyte is ordinarily rather broad but as the chromosomes begin to pull apart, it becomes in many cases, long and slender. The fibers of the spindle converge at the poles, but no centrosomes could be found in any of these stages. This may have been due to the stains used. The chromosomes in the metaphase plate do not divide synchronously. The accessory tends to divide precociously (Figs. 8 and 9) and is generally followed by the smallest chromosome (Figs. 6 and 7). As the accessory advances toward the poles,

the larger chromosomes are seen to be pulled apart. As they divide and separate they are usually still connected by two thick threads (Figs. 6, 7, 9). These fibers stain deeply with hæmatoxylin but are lost sight of as the chromosomes are pulled farther apart and the cell wall begins to constrict. There is no sign of a zwischenkörper as described by Paulmier for Anasa. Each of the two resulting secondary spermatocytes (Figs. 11, 12, 13) contains six chromosomes in the form of dyads and one monad, the accessory. There is no trouble identifying the accessory because of its size and position.

Second Spermatocytic Division.

The second division follows directly upon the first without any resting stage. In the equatorial plate of the second division the chromosomes are arranged in the characteristic ring of six with the smallest chromosome in the center. In the case of *Anasa tristis* Paulmier finds no such regular arrangement though he says the small chromosome generally lies in the center. The chromosomes now arrange themselves on the middle of the spindle and very soon the accessory is seen to leave the group and pass undivided in advance of the others toward one of the poles (Figs. 14, 16, 17, 18, 19, 20). In *Anasa* the accessory lags, and is not drawn toward one of the poles until the other chromosomes have practically come to the end of their movement. The remaining dyads then divide apparently in the same order as for the first division, the smallest leading (Figs. 15, 17, 19). These then pass to opposite poles and the cell constricts in the middle. The resulting cells are smaller than those of the first division, half of them containing seven chromosomes, and half six (Figs. 21, 22, 23, 14). The chromosomes of these spermatids have the same characteristic arrangement as those of the primary and secondary spermatocytes, one half of them having a ring of six with a small one in the center and the other a ring of five with the small one also in center. There is a typical massing of the chromosomes in the spermatid before the nuclear wall is formed.

The spindle of the second spermatocyte is similar in shape to that of the first, being rather broad and thick at the initiation

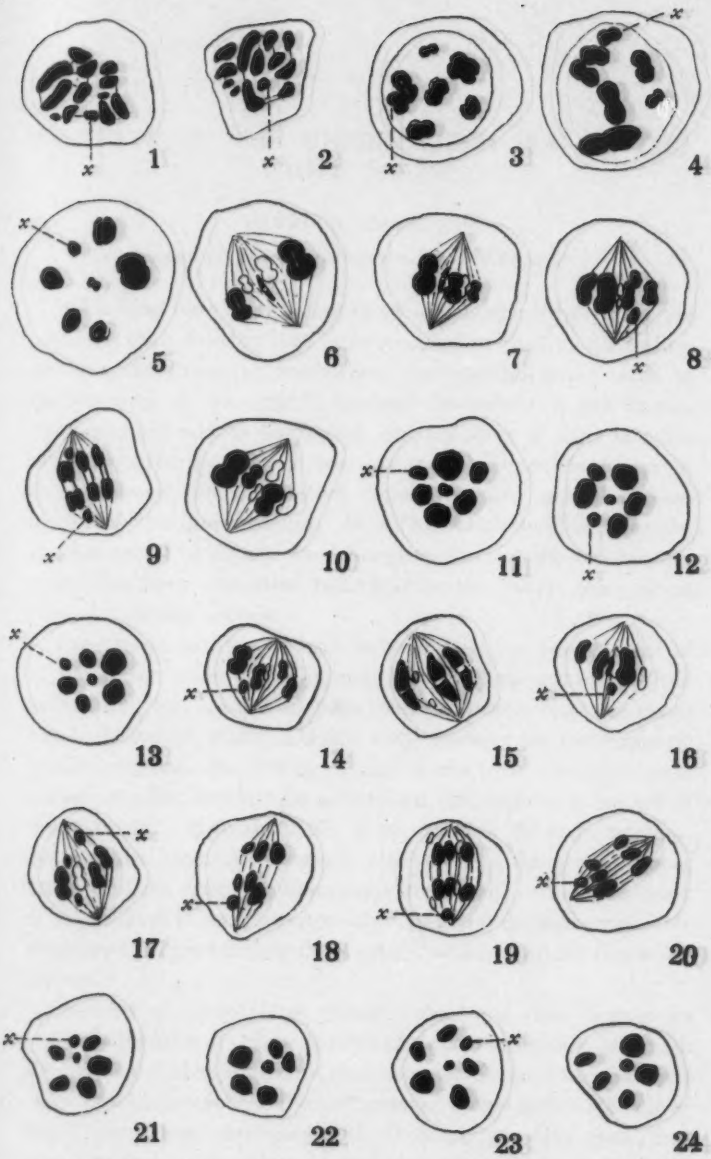
of division, and as the chromosomes are pulled apart, taking on a slender elongated appearance.

In conclusion it may be suggested that the female has two X-chromosomes, or fourteen in all. Wilson reports that *Leptocoris trivittatus* has fourteen in the female and thirteen in the male.

Explanation of Plate I.

All drawings were made with the aid of a camera lucida at table level. A Zeiss microscope with compensating 12 ocular and 2 mm. oil immersion objective was used. The drawings have been reduced one third off.

1, 2. Equatorial plate of spermatogonia.
3, 4. Tetrad stages, showing all chromosomes.
5. Polar view of first spermatocyte.
6–8. Side view of same.
9. Early anaphase of first division.
10. Side view of first spermatocyte division.
11–13. Polar view of second spermatocyte.
14–20. Second spermatocytic division.
21, 22. Spermatids, corresponding daughter cells.
23, 24. Spermatids, corresponding daughter cells.



LELIA T. PORTER.

THE FATE OF THE UNFERTILIZED EGG IN THE WHITE MOUSE.

HARRY H. CHARLTON,

OSBORN ZOÖLOGICAL LABORATORY, YALE UNIVERSITY.

It has long been known that in mammals many more eggs are ovulated than develop into embryos, due in part to the failure of the spermatozoa to reach them, and perhaps more often to the inability of the eggs to implant themselves in the uterus. Such eggs are said to degenerate, but the story of their fate has been recorded in only a few instances. Since ovulation in the mouse is independent of copulation, this animal was selected for the investigation. It is natural to suppose that the process would be similar to the degeneration of the ovarian egg which has been prevented from leaving the ovary, the process termed follicular atresia.

The atresia of ovarian eggs in mammals has been described in detail by many investigators, but the degeneration of the unfertilized egg in the Fallopian tube and uterus has been given but little special study. Heape ('05) working on the domestic rabbit, mentions the finding of eggs in the tube which for some reason or other had not been fertilized and were in a process of degeneration. Hartman ('16), in his study of the early development of the American opossum, also refers to the great number of unfertilized eggs, approximately 50 per cent., which he found in the uterus, in an earlier or later stage of degeneration. He ventures the opinion that these eggs would eventually leave the uterus.

Students of atresia have usually recognized that there is an early disturbance of the nucleo-cytoplasmic relationship, although they have not always given it that name, and that it was followed by a rearrangement of the cytoplasm. Some writers however insist that a true parthenogenetic cleavage may take place, and continue in a more or less normal manner up to, in some cases,

the ten or twelve cell stage before the degenerative processes get the upper hand.

The purpose of the present investigation is to describe step by step the degeneration, whether it be fragmentation or parthenogenetic development, of the unfertilized egg in the white mouse, and to compare the process with follicular atresia in the mouse and other mammals.

The work was undertaken at the suggestion of Prof. W. R. Coe, and done under his supervision. I am pleased to acknowledge his many valuable suggestions during the course of the investigation.

LITERATURE.

Kingery ('14) describes the method of atresia in the follicles of young mice. In his opinion the process is one of degenerative fragmentation only, that the spindle fibers of the second maturation spindle break, forming aster-like radiations and that later the achromatic fibers entirely disappear. Then the loose chromosomes form vesicles, and occasionally these fuse together to form larger vesicles. This disturbs the nucleo-cytoplasmic relationship, and an attempt to counter balance is made by the cytoplasm breaking up and surrounding the nuclei, the larger amount of cytoplasm enclosing the larger nuclear vesicles. In some cases the cytoplasmic fragments have no nucleus whatever. These fragments with or without nuclei are later absorbed, probably by the action of phagocytic cells of follicular origin.

Newman ('13) found that in the armadillo the first maturation spindle was formed in the ovary and that the egg then waited for ovulation. Eggs not located near the periphery of the ovary were not ovulated. In such cases Newman found that only a small percentage cut off the first polar body and that only three per cent. in several hundred cases gave off the second polar body, while ninety per cent. of the eggs were struck by a process of follicular atresia, which is either a cytolysis or a parthenogenetic development. He considers only the latter contingency. In such eggs there is first a casting out of the deutoplasmic mass from the formative cytoplasm. This he considers to be an act of rejuvenation on the part of the dying cell. Released from the burden of the yolk the cell is better

able to carry on the natural processes. The abstrictions of the yolk often look like multicellular structures, but Newman thinks this appearance is due to fixation. Some of these yolk fragments which he calls cytoids contain deutoplasmic granules.

Newman finds numerous tri- and multipolar spindles. These, he thinks, would result in nests of nuclei without division of the cytoplasm. Sometimes instead of a single resting nucleus formed from the maturation spindle, two nuclei might be formed instead, without the extrusion of the polar body, and suggests that from these double nuclei, the multipolar spindles might be formed. As the number of chromosomes is more than the haploid number such spindles cannot be maturation spindles. In those cells with a true cleavage spindle, Newman is inclined to the view that no polar bodies have been given off. The process is in his opinion a true parthenogenesis, but development does not proceed beyond the eight cell stage and even at that time advancing degenerative processes are to be seen.

Heape ('05) found that in the domestic rabbit copulation acted as a stimulus to certain internal rearrangements which ended in severing the ovum from its source of nourishment. At about nine hours after copulation the two polar bodies are formed and ovulation takes place about an hour later. He says: "Once freed from the ovary the mature ovum is incapable of assimilating nutriment unless it be fertilized; if from any cause fertilization is not effected, the ovum quickly dies, although it is bathed in the nutrient material supplied by the maternal tissues; ova thus degenerating are from time to time to be seen in the Fallopian tubes." It is therefore necessary for spermatozoa to be at the top of the Fallopian tube because, unlike the condition in the mouse, the ovum is dehisced from the ovary without any discus cells to provide it with food.

Bonnet ('00) reviews the different theories of degeneration and takes the view that the various spindles figured by the exponents of the parthenogenetic idea are to be considered more or less abnormal maturation spindles, and not cleavage spindles.

Van der Stricht ('01), in his study of follicular atresia in the bat, comes to the conclusion that the oöcyte of the second order forms a cleavage spindle and divides parthenogenetically. He

has traced an apparent normal cleavage as far as the six-cell stage. He also described multipolar spindles and occasionally two spindles in the same egg. The first polar body is occasionally seen to divide.

Kirkham ('07) gives an excellent review of the literature on the early development of mammalian eggs previous to 1907. The common occurrence of spindles in the polar body led him to conclude that the polar body would divide mitotically. Abnormal eggs containing tripolar spindles and in one case an egg containing two spindles were observed. Kirkham agrees with Sobotta ('95) and Rubaschkin ('06) that an egg never develops after the formation of the first polar body and the second polar spindle unless fertilization takes place, the egg degenerating within the ovary or in the Fallopian tube.

Athias ('09) also takes the view that the process is entirely one of degenerative fragmentation.

Material and Methods.

The mouse is an animal well adapted to such a study, for we know from the investigations of Sobotta, Kirkham, and later of Mark and Long, that ovulation takes place without the necessity of copulation in from thirteen and three-quarter to twenty-eight and one-half hours (Mark and Long) after parturition. The sexes were kept together in suitable cages and the females examined frequently for signs of pregnancy. Whenever a female was found to be pregnant she was placed in a cage and examined each morning. As soon as a litter was found it was removed, and a record of the time made. It is from this time record that all the ages for the various eggs are figured.

Animals were killed at intervals varying from one and one half days to four days and nine hours after the finding of the litter. The body was immediately cut open and the ovaries with tube and uterus removed and placed in the fixing solution. For this purpose Zenker with acetic, and Carnoy's 6–3–1 solution, were found to be about equally good. For tube eggs strong Flemming proved excellent. The prepared sections were stained in Heidenhain's iron hæmatoxylin, Ehrlich's hæmatoxylin, or Flemming's Triple stain, usually with suitable counter stain.

Observations.

Thirty-six hours after finding the litter, eggs are almost certain to be found in the upper part of the Fallopian tube. They always show the second maturation spindle and may or may not have the polar body attached. If present, the polar body has the chromosomes arranged in the form of a spindle (Fig. 1.) During the next twelve hours there is a change in the polar bodies. The chromatin of the spindle breaks down and arranges itself into one, two or many resting nuclei. The formation of two nuclei is well illustrated in Fig. 2. Here the vesicles have been formed but a number of chromosomes or chromatin bodies have not yet entirely lost their individuality. To one side of the polar body a small body, the first indication of a mitochondrial substance, may be seen. Fig. 3 shows a later stage in which the chromatin of the polar body has formed eight vesicle-like nuclei. Since eggs with three or more polar bodies are not infrequent (see Fig. 4), one is led to believe that the cytoplasm segments, forming itself around the various vesicles. Long and Mark ('11) find that the first polar cell often divides amitotically. They consider that this aids the degeneration and absorbtion of the polar bodies due to the increase in the exposed surfaces. Kirkham also, in work which has never been published, has observed that the first polar cell occasionally divides forming a number of fragments.

The achromatic fibers of the second maturation spindle disappear early, since in only a few cases have they been noted in eggs as old as forty-eight hours. The spindle stands out clear and distinct with the chromosomes in their natural relations but no signs of fibers can be observed.

Between forty-eight and seventy-two hours a renewal of activity begins in the unfertilized egg. Its effect is first apparent in the second maturation spindle, which may break down and form a single resting nucleus. Three eggs from a seventy-six hour mouse all have a single nucleus both in the eggs and in their polar bodies. More often however numerous vesicles instead of one result from the breaking down of the spindle. An examination of Fig. 5 will make it clear how this takes place. This egg is from a mouse in which one of the uterine horns was

closed by a tumor, thus preventing access of the sperm to the eggs on that side. The mouse was killed sixty hours after finding the litter. In the tube on the occluded side, two eggs were found, one in the second maturation spindle stage, and the other showing the breaking down of the spindle. In the other horn fertilization had taken place and the eggs were in the four-cell stage. In the figure it will be noted that the chromatin of the spindle has for the most part gone into the formation of a circular vesicle, in which a number of chromosomes remain practically unaltered.

A later stage picturing the breaking down of the spindle is that of Fig. 6. Here the lobes are larger and have become separated from the central vesicle. All the chromatin is now found in the walls of the various vesicles, none remaining as chromosomes.

The loss of the achromatic fibers and the breaking down of the spindle is probably due, as suggested by Kingery ('13), to the degenerative changes appearing quite early in the cytoplasm. This is shown by the fact that the cytoplasm stains more deeply than in normal eggs. Numerous dark staining granules are seen, particularly near the periphery of the egg. These constitute the mitochondrial bodies.

Kingery ('13) is of the opinion that the spindle fibers of the second maturation spindle break and free the chromosomes, which then migrate into the cytoplasm and form numerous nuclear vesicles. In my material the achromatic fibers disappear early, and from Figs. 5 and 6 it would appear that the vesicles may be formed *in situ*, and not necessarily from chromosomes which have wandered from the place of the original spindle.

In Figs. 7, 8 and 9 a different process of spindle disintegration is seen. These three eggs are all from the same mouse and were located close together in the lowest portion of the tube very near the uterus. From Fig. 7, it is evident that while the spindle fibers have disappeared; the chromosomes, although contracted and hugged closely together, still retain the form of a second spindle. Several chromatic fragments have evidently broken off and are seen scattered in the cytoplasm. The succeeding stage may be noted in Fig. 8. The individual chromosomes

have separated from their spindle position and lie scattered in the cytoplasm. One of the chromosomes shows the beginning of vesicle formation, which is seen completed in Fig. 9. Fig. 11 shows essentially the same condition. The polar body has divided and several small phagocytes may be seen just inside the zona. In Fig. 10 the chromatin is arranged around the periphery of the vesicle and not clumped to one side as in Fig. 9. One notices quite a similarity between these vesicles and those in process of formation in Fig. 6, and suggests that they may have been preceeded by such a stage.

After the formation of these numerous nuclear vesicles, the fragmentation of the cytoplasm takes place. Figs. 16 and 17, adjacent sections of a seventy-two hour egg, indicate that the fragmentation may take the form of a protoplasmic budding. The large cell shown in the figure has constricted off two fragments and is forming others. Adjoining sections show five additional fragments, making seven in all. Nuclear material is present in only two of the seven fragments. Of course one cannot say that all such fragments are constricted off from the egg, for there is always the possibility that some of them may have come from the polar bodies. It has been shown by Hartman ('16) in the opossum, and by Newman for the armadillo that normally one of the first activities of the unfertilized egg is the unburdening itself of the deutoplasmic material. This is done without assistance of any spindle or apparent nuclear influence. While it cannot be said that the fragments budded off in the mouse are exclusively of yolk material, the process is essentially a similar one.

Figs. 13, 14 and 15 are later stages in the fragmentation process. They represent what Kingery ('13) calls the "morula" stage. Figs. 14 and 15 represent two adjoining sections of the same "morula," which consists of about twenty-one cytoplasmic fragments.

We have seen how the second maturation spindle breaks down and forms either a single resting nucleus or numerous nuclei, and have traced the latter to the "morula" stage. When, on the breaking down of the spindle the egg is able to form a single or only a few resting nuclei, it would seem to indicate exceptional

vitality and a close approximation of the normal condition. In such an egg the process of atresia would naturally be somewhat delayed. The egg with but one or two nuclei does not fragment immediately, and it is possible that such eggs may pass from the body, as suggested by Hartman ('16) for the unfertilized egg of the opossum. This possibility is well illustrated in Fig. 18, which shows an unfertilized egg one hundred and five hours after finding the litter. Only two nuclei are present. The mitochondrial bodies (*b*) can be distinguished from the nuclei by their peculiar crystalline appearance and elliptical shape. Although these bodies are found in most of the fragmented segments to be described later, they are smaller and stain less intensely in hæmatoxylin. They are surrounded by a light area which gives them the appearance of having shrunken from the cytoplasm. In Fig. 19 the mitochondrial bodies are seen to be smaller but more numerous. In the two upper fragments they did not cut cleanly but were pushed aside by the knife. This would seem to indicate that they were much more compact and dense than the surrounding cytoplasm.

Hartman ('16) finds a similar structure in the egg of the opossum, and describes it as a homogeneous, non-granular body staining pink or lavender in iron hæmatoxylin, and bordered by a light-colored band.

The multinucleated eggs soon break up into a number of fragments, often forming two or more parts very nearly of the same size and often appearing like normal cleavage stages, were it not for the presence of extra nuclei. Figs. 12, 13 and 14 show different stages in this process.

The ultimate fate of the fragmented egg will now be considered. Very early in the degenerative process, phagocytic cells are found inside the zona pellucida, between the cytoplasm and the zona. It is impossible to mistake these cells for fragments of either the egg or polar bodies for they appear almost like transparent vesicles with the deep staining nuclear material either scattered as granules or irregularly clumped in a way which immediately suggests their origin as polymorphonuclear leucocytes. Kingery described similar cells but thought them of follicular origin. The number of these phagocytic cells inside the zona varies,

eight or ten being the largest number found. These cells probably aid in the disintegration of the periphery of the cell. This process may begin some time before fragmentation sets in, as is evidenced by a study of Fig. 9. Here the space at one end of the egg filled with dark granules indicates that the cytolysis of the cytoplasm has begun.

The zona persists in nearly all cases as far as the eggs have been followed. Fig. 6 shows one of the exceptions, for here the zona is gone and many dark staining fragments which may have come either from the polar body (which is now very small) or from the broken down zona, are seen surrounding the egg. Several phagocytic cells, one of which is figured, are to be seen among the fragments. Although the zona is present in almost all cases, a careful examination usually brings to light several breaks, and it is probably through these that the phagocytic cells are able to reach the egg.

Figs. 19 and 20 show the last stages in the disintegration and absorbtion of the fragments by the phagocytes. Some of the fragments appear as mere shells with only occasional granules, while the outer edge of others shows a honeycombed condition due to excessive vacuolization.

It is found that the unfertilized egg completes its passage through the Fallopian tube and enters the uterus about the end of the third day or almost at exactly the same time as given by Sobotta for the normal segmenting egg.

Smith ('17), basing ovulation as occurring twenty hours after parturition, finds that unfertilized eggs of the mouse enter the uterus about 76 hours after ovulation and are found in the last fold of the uterus at 88 hours.

The final dissolution of the fragmented egg evidently is completed early on the fourth day, since out of eleven mice older than four days, all showing corpora lutea in the ovaries, indicating that ovulation had taken place, in only three could eggs be found. A single egg was found in the uterus of a mouse one hundred and five hours after parturition (Fig. 18).

In not a single case has anything approaching a cleavage spindle as described in atresic ova by Spuler, Loeb or Newman, been observed, but it is not to be interpreted that they may

not occur, because cleavage spindles are so short lived that the study of a much greater number of eggs would be necessary before coming to any such conclusion.

SUMMARY.

The cytological changes which the unfertilized egg of the mouse undergoes in the Fallopian tube and uterus are closely similar to those which occur in the eggs of atresic follicles in the ovary.

The processes are considered degenerative and have only a superficial resemblance to parthenogenetic development.

The breaking down of the second maturation spindle of the unfertilized egg usually results in the formation of several or many nuclei. Rarely a single nucleus is formed, in which case the egg does not fragment rapidly and may pass from the uterus before the degenerative processes are complete.

The eggs with many nuclei divide into numerous cells, of which some are provided with degenerating nuclei. These are further disintegrated and absorbed by phagocytic cells, which make their way into the egg probably through breaks in the zona. The phagocytes from their appearance are polymorphonuclear leucocytes, and they evidently act on the cytoplasm causing a vacuolization of its outer portions and a later crumbling of its periphery; the end being a complete disintegration of the egg and its absorption by the phagocytes.

LITERATURE.

Athias, M.

'09 Les phénomènes de division de l'ovule dans les follicles de Graaf en voie d'atrésie chez le Lerot (Eliomys quercinus L.). Anat. Anz., Bd. XXXIV. No. 1.

Bonnet, R.

'00 Giebt es Wirbeltieren-Parthenogenesis? Ergebn. der Anat. u. Entwickl., Bd. 9.

Heape, W.

'05 Ovulation and Degeneration of the Ova in the Rabbit. Proc. R. Soc. London, Vol. 76b.

Hartman, C. G.

'16 Studies in the Development of the Opossum Didelphys virginiana. Journ. Morph., Vol. 27, No. 1.

Henneguy, L. F.

'94 Recherches sur les follicules de Graaf chez les mammifères et quelques autres Vertébrés. Journ. d. anat. et d. Physiol., Ann. XXX., No. 1.

Huber, G. C.

'15 The Development of the Albino Rat, Mus norvegicus albinus. I., From the pronuclear stage to the stage of mesoderm anlage; end of the first to the end of the ninth day. II., Abnormal ova; end of the first to the end of the ninth day. Journ. of Morphology, Vol. 26.

Kingery, H. M.

'13 So-called Parthenogenesis in the White Mouse. BIOL. BULL., Vol. 27.

Kirkham, W. B.

'07 The Maturation of the Mouse Egg. BIOL. BULL., Vol. 12, No. 4.

'07a Maturation of the Egg of the White Mouse. Trans. Conn. Acad. Arts and Sci., Vol. 13.

Loeb, L.

'01 On the Progressive Changes in the Ova in Mammalian Ovaries. Journ. of Medic. Research, Vol. 6.

'05 Ueber hypertrophische Vorgänge bei der Follikelatresie, nebst Bemerkungen über die Oöcyten in den Marksträngen und über Teilungserscheinungen am Ei im Ovarium des Meerschweinchens. Arch. f. mikr. Anat., Bd. 65.

'11 The Parthenogenetic Development of Ova in the Mammalian Ovary and the Origin of Ovarian Teratoma and Chorio-epitheliomata. Journ. Amer. Medic. Assoc., Vol. LVI.

'12 Ueber chorionepitheliomartige Gebilde im Ovarium des Meerschweinchens und über ihre wahrescheinliche Entstehung aus parthenogenetisch sich entwickelnden Eiern. Zeitsch. f. Krebsforschung., Bd. 11, No. 2.

Long and Mark.

'11 The Maturation of the Egg of the Mouse. Carnegie Institute Publication, No. 142.

Newman, H. H.

'13 Parthenogenetic Cleavage of the Armadillo ovum. BIOL. BULL., Vol. 25.

Rubaschkin, W.

'06 Ueber die Veränderungen der Eier in den zugrunde gehenden Graafschen Follikeln. Anat. Hefte, H. 97.

Smith, H. P.

'17 The Ovarian Cycle in Mice. (Abstract.) Anat. Record, Vol. 11.

Sobotta, J.

'95 Die Befruchtung und Furchung des Eies der Maus. Arch. f. Mikr. Anat., Bd. 45.

Spuler, A.

'00 Ueber die Teilungserscheinungen der Eizellen in degenerierenden Follikeln des Säugerovariums. Anat. Hefte, H. 50.

Van der Stricht, O.

'01 L'atrésie ovulaire et l'atrésie folliculaire du Follicule de de Graaf, dans l'ovaire de Chauve-Sauris. Verhandl. d. Anat. Gesellsch., 15 Versamml., Bonn.

EXPLANATION OF PLATES.

All the drawings are from sections of unfertilized eggs in the Fallopian tube or uterus. They were made with the aid of a camera lucida, and with the exception of Fig. 5 are reproduced at a magnification of 900 diameters.

Abbreviations.

a, zona. *b*, mitochondrial body. *c*, phagocytes. *d*, vacuole. *e*, degenerating protoplasm.

Plate I.

Fig. 1. (48 hours.) Egg with the second maturation spindle. Polar body shows chromosomes arranged on the spindle fibers. Eggs of this type, showing no changes are found in the tube up to forty-eight hours after parturition.

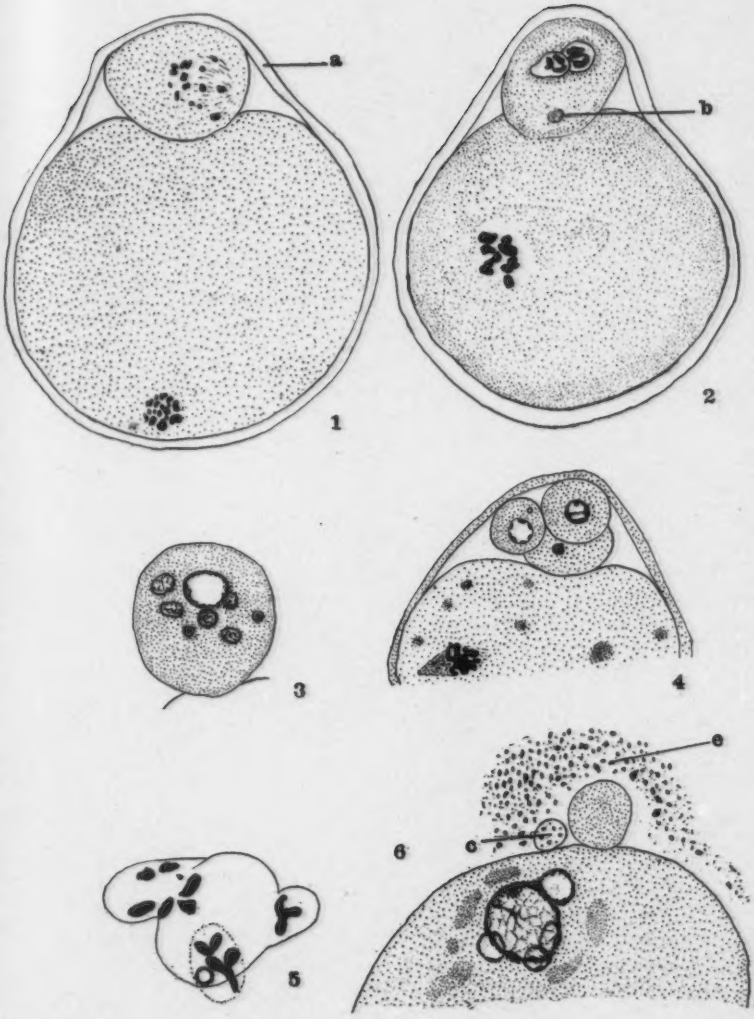
Fig. 2. (56 hours.) In this egg the spindle is present but the chromosomes seem to have clumped together more than in Fig. 1. The spindle of the polar body has disappeared and in its place are seen two resting nuclei.

Fig. 3. Polar body (60 hours) from same mouse as Fig. 5 showing many resting nuclei formed from the breaking down of the polar spindle or by the chromatin fragments often observed in the polar body.

Fig. 4. (48 hours.) Showing the fragmentation of the polar body. Here the first polar body has divided once and one of the parts has redivided. The spindle cut somewhat obliquely is one of few showing good achromatic fibers. These fibers seem to end in a centrosome-like body.

Fig. 5. (60 hours.) Chromosomes of the second maturation spindle breaking down and forming resting nuclei. Vesicular lobes are seen extending out from the main vesicle. Quite a number of the chromosomes have not yet entered into the process and are seen in the lobes of the forming nuclei. This mouse had a tumor in one of the uteri which prevented fertilization, while on the other side, eggs in the tube had reached the four cell stage. (× 1,800.)

Fig. 6. (78 hours.) A later stage of vesicle formation from the maturation spindle. Chromatin all in the vesicles, no chromosomes as such. This was one of the few eggs in which the zona was missing. Many dark staining granules surrounded the egg and in among them several phagocytes. One phagocyte shown at left of polar body, which is much smaller than normal, suggesting that the phagocytes have been acting upon it and probably upon the zona also.



H. W. CHARLTON.

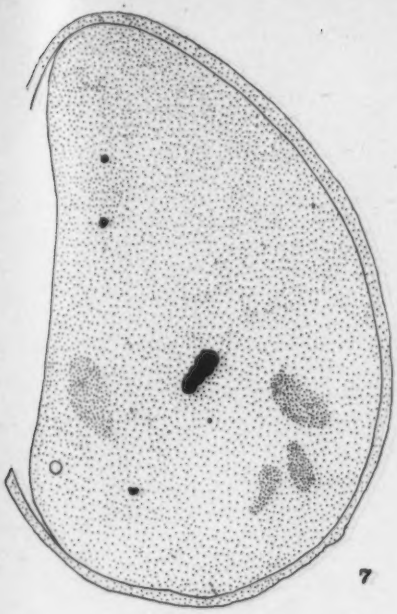
PLATE II.

FIG. 7. (72 hours.) Spindle fibers missing, chromosomes of spindle hugged closely together. Several parts separated and out in the cytoplasm.

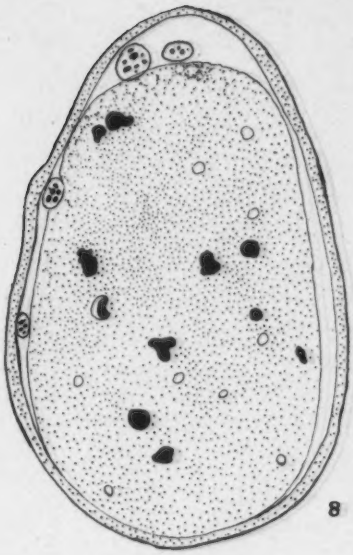
FIG. 8. (72 hours.) A later stage of the above. Chromosomes scattered in the cytoplasm. One showing the beginning of vesicle formation. Several phagocytes are present inside the zona. Cytoplasm near open end shows beginning of disintegration.

FIG. 9. (72 hours.) Completion of vesicle formation begun in Fig. 8. The chromatic material clumped to one side of the vesicle. Quite a few small particles of disintegrated cytoplasm may be seen inside the zona indicating the activity of the phagocytes.

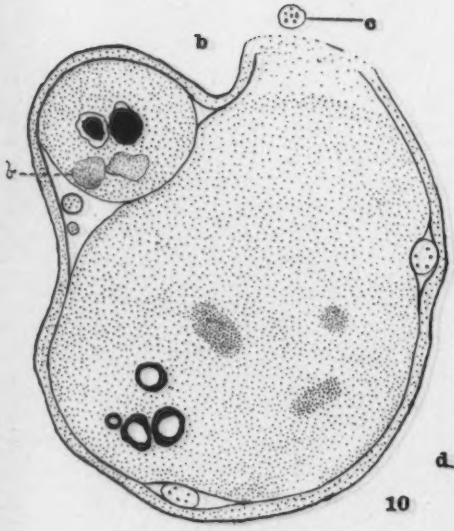
FIG. 10. (72 hours.) Reconstruction showing ring-like formation of nuclear material. Possibly a succeeding stage to Fig. 6. A phagocyte cell is noticed just outside the zona, which is very faintly stained.



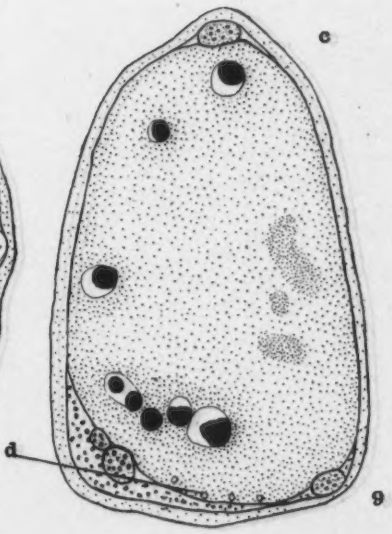
7



8



10



9

H. W. CHARLTON.

PLATE III.

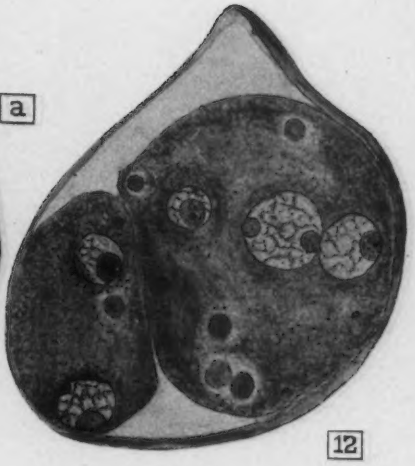
FIG. 11. (72 hours.) Egg showing four resting nuclei, and a number of mitochondrial bodies. In addition a divided polar body and two or more smaller structures which are probably phagocytic cells.

FIG. 12. (72 hours.) Fragmented egg looking very much like a normal two-cell stage, except that each blastomere has two or three nuclei. The nuclei contain nucleoli and appear perfectly normal.

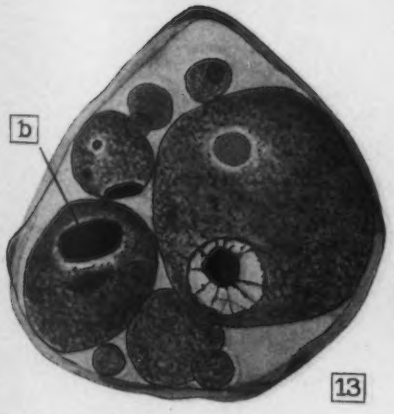
FIG. 13. (81 hours.) A later stage of fragmentation. A large resting nucleus is seen in one of the larger segments, while other fragments have none. In two of the cells the mitochondrial body is quite conspicuous.

FIG. 14. Egg of uncertain age, consisting of twenty-one fragments. Numerous phagocytic cells are seen in and about the fragments. Zona entirely missing.

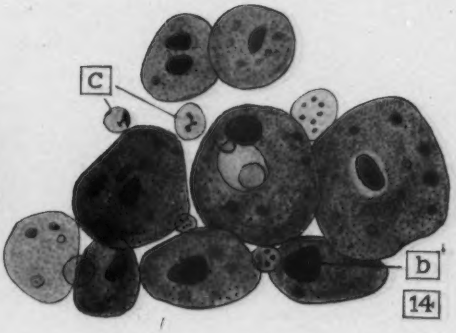
11



12



13



14

H. W. CHARLTON.

PLATE IV.

FIG. 15. Drawing of adjacent section to that shown in Fig. 14.

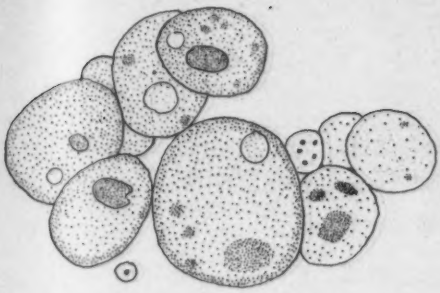
FIG. 16. (72 hours.) Egg showing the formation of protoplasmic fragments independent of any spindle. These are later pinched off and form small segments inside the zona.

FIG. 17. Drawing of adjacent section to that shown in Fig. 16. A study of all the sections of this egg shows that seven fragments have been pinched off, and that of these only two appear to contain nuclear material.

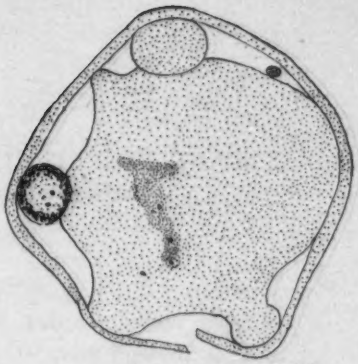
FIG. 18. (105 hours.) Egg is without zona, contains two nuclei and two immensely large refractive mitochondrial bodies. The polar body still remains attached to the egg.

FIG. 19. An 81-hour egg showing phagocytic effect. In two upper cells the mitochondrial bodies have been pulled out of the cytoplasm towards the left by the knife, leaving clear spaces where they originally were, indicating the solid character of these structures. Many vacuoles are seen around the border of the fragments. Between the cells dark stained particles of degenerated cytoplasm is being absorbed by the phagocytes.

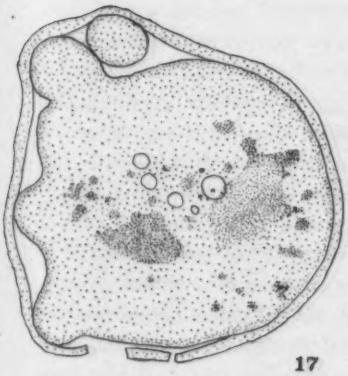
FIG. 20. (81 hours.) A later stage than Fig. 19. In one of the fragments hardly any content is to be seen except a few scattered granules.



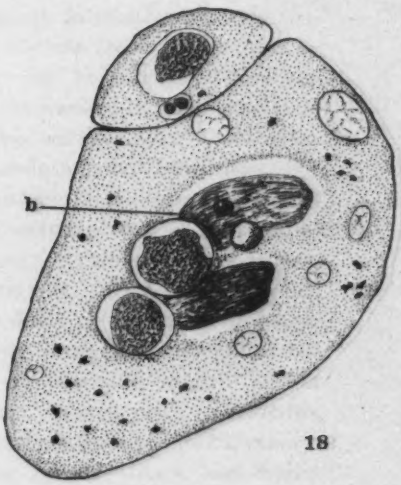
15



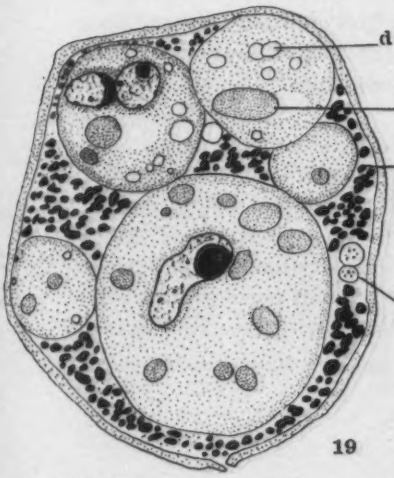
16



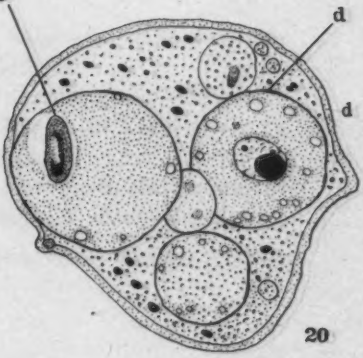
17



18



19



20

H. W. CHARLTON.

OBSERVATIONS ON THE STRUCTURE OF DOUBLE MONSTERS IN THE EARTHWORM.[1]

ROXIE A. WEBER.

I. Introduction.

The work which has thus far been done on double monsters in the terricolous group of annelids is quite limited, since in only a few species, *Lumbricus trapezoides*, *Lumbricus terrestris*, *Allolobophora fœtida* and *Allolobophora trapezoides*, has this condition been observed. The first of these forms is described by Kleinenberg, the remaining three by Vedjovsky. These two investigators agree neither as to the method of union nor as to the cause of this phenomenon. It is not the object of this paper to give the factors influencing such monster formations, but merely to describe the manner in which the members are joined.

Material and Technique.—The material studied consists of twelve double embryos, the shortest member of which is at least 60 segments in length, the longest 125, one monster in which one individual consists of 16 the other 17 segments, 19 double gastrulæ and two embryos showing a bud on one side of the blastopore, and one case of an egg consisting of two distinct hemispheres connected by a band of large cells. The entire supply was obtained from Professor Patterson, of this University.

The fixing fluids employed were Meves, Gilson, and Bouin, all of which gave only fairly good results. Ehrlich's hæmatoxylin was used as a stain.

The species studied is *Helodrilus caliginosus trapezoides*, according to Professor F. Smith's identification.

Review of the Literature.—Before describing the individuals studied, it will perhaps be well to give a brief review of the results of the two authors above mentioned.

Kleinenberg found that the number of eggs in the capsules of *L. trapezoides* varies from three to eight of which usually one,

[1] Contributions from the Zoölogical Laboratory of The University of Texas, No. 139.

though sometimes two or three, produces an embryo. The remaining ones, not becoming fertilized, disintegrate completely.

By a series of somewhat irregular cleavages the egg develops into a germinal bladder, one layer in thickness (Pl. IX., Fig. 2, Kleinenberg). Thereupon a two-layered condition begins to appear, one side of the cell mass becoming differentiated in advance of the other. At this pole all the cells divide rapidly except two, which become pushed in and covered over by the small blastomeres. From these two cells (mesoblasts) are derived the first rudiments of entoderm and mesoderm (Pl. IX., Fig. 4).

While this elongation is taking place a transverse furrow appears midway between the two ends extending almost entirely around, leaving the two hemispheres connected by only a few enlarged ectodermal cells.

When this stage has been reached the cells of the other pole begin to undergo the same changes and finally we have formed from each half an embryo joined in varying degrees to the other by a band of ectodermal cells. The separation is eventually effected by a series of rotations which usually result in the breaking of the uniting cord. When for one reason or another this is not accomplished we have true monstrosities in all degrees of coalescence.

According to Kleinenberg the union never extends to the internal organs but is confined to the external epithelium of the body wall.

The monsters formed in this manner are of the same or nearly the same size. There is however still another type of abnormality (Pl. IX., Fig. 10), *e. g.*, those showing bud formation. Kleinenberg explains their production as being due to a very unequal development of the two halves of the above mentioned cell mass.

In all his work he has found only a few cases in which two individuals did not emerge from one capsule and in those few exceptions rudiments of a second were usually found. For this reason he concludes that each egg produces two individuals normally.

Vedjovsky on the other hand holds this condition to be ab-

normal. He explains the formation of double embryos as being due to what he terms *Doppelfurchung*. The egg elongates in the direction of the animal pole and subsequently divides into two blastomeres either of equal or unequal size. They are designated as A and a. From each of these cells there is produced a quartet, one lying upon the other giving the appearance of micro- and macromeres in the cleavage of *Crepidula* or the eight cell stage of *Synapta*, depending on the proportionate sizes of A and a. Each of the quartets finally gives rise to an individual which remains attached to its companion. If the a cell is smaller than A, there is produced one well developed individual and a bud.

This author has found examples of monster formation in *Lumbricus terrestris*, *Allolobophora fœtida* and in *Allolobophora trapezoides*. In the first group he found two cases in both of which the two individuals were joined on the dorsal side through one segment. Only one monster was found among several hundred embryos of the second group. In the third group a large number was found which the author describes under three divisions.

A. Those monsters in which the individuals are united on the ventral side along the entire length of the body (Pl. 19, Fig. 14).

B. The cases in which the members are joined on the dorsal side (Plate 21, Fig. 7).

C. The double monsters in which the individuals are fused end to end. (*a*) Those in which both members are of similar length (Pl. 21, Fig. 9). (*b*) Those cases in which one member is rudimentary (Pl. 19, Fig. 12).

II. Observations.

Even though these two workers differ so markedly in their conclusions it will be noted from the following that the observations made by the writer are similar in many respects to those made by both of these men.

In this study of the monsters of *Helodrilus caliginosus trapezoides* it has been found that the greatest variety exists in the structure of the nervous system. There is in every case a complete union of the five layers of the body wall, and the

digestive tract is either fused or not fused, but the nervous system is modified in a variety of ways. The manner and extent of union of these individuals and their organ systems are best described in the following groups.

1. Those double monsters in which the union is dorsal. This group may again be subdivided into—

(*a*) Those in which the union extends to the alimentary tracts. Of these there are two examples, Nos. 95 and 171, Plate I. From Figs. 1 and 2, Plate I., it can be seen that the fusion in No. 171 is through one segment only, while in No. 95 it extends through five. A description of sections through this monster will serve to show the general relationship of the organ systems in the members of this group. Fig. 3, Plate I., is a section through the anterior portion showing the condition of the nervous system. It will be seen that there is almost a continuous band of nerve tissue extending around the pharynx. A study of the neighboring sections shows that this band is really complete. It will also show that the portion marked (*b*) in the figure enlarges into a bilobed brain and that a similar structure exists on the opposite side of the pharynx. At the same time there is also to be found at each end of the greatly elongated pharynx another set of ganglia. It would be impossible here to determine which are brain and which are ventral cord ganglia were it not for the presence of setæ (*s*). This at once leads to the conclusion that the ganglia at the end of the pharynx must be those of the ventral cord. This inference is further proven by a study of sections through a more posterior region, as shown in Fig. 4, Plate I. Here are no longer to be found the ganglia on either side of the pharynx but only those at the opposite ends. This section too shows the setæ in normal relationship with the ventral cord and the nephridæ in their natural position. They are shown only on one side but those of the other half can be seen in the next section.

It will be noted that the single pharynx is greatly elongated from end to end of the monster. Throughout the fused part it remains as one cavity dividing into two parts only when the point of separation of the two members is reached, one passing into each individual. Surrounding the pharyngeal cavity there

is a great mass of muscular tissue attached by thin strands to the body wall. This condition is exactly similar to that found in single individuals.

The sub-intestinal blood vessels are clearly visible (*v.b.v.*) in their normal position between the alimentary tract and the nerve cord. On each side of the muscular pharynx there lies a more or less regular vessel (*D.B.V.*) which when traced to the point of separation of the two members will be found to approach the center and then one passes into one individual, the other into the other, the vessel on the left side passing into the upper half, the one on the right into the lower half.

Though the other monster is formed by a coalescence of the two members through one segment only, that union is complete in every respect from body wall to pharynx. Fig. 5 is a longitudinal section through No. 171 showing a portion of one of the brain ganglia together with a small part of the nerve cord of each of the individuals. A study of the successive sections will show that the pharynx arises from a flattened portion which is lined with the same kind of epithelial cells as the rest of the digestive tract and which lies between the two members as indicated in Fig. 2, Plate I. (*m*). The ventral cords are found to be connected to the cerebral ganglia, of which there are two sets, one on each side of the mouth opening, by means of commissures as in No. 95.

The setæ, not shown in this figure, and the nephridæ lie along the same side of the body as the nerve cord.

(*b*) Those in which the alimentary tracts have not become united. Nos. 91, 92, 142 and 173 shown in Plate II as Figs. 6, 7, 8 and 9 illustrate this group. It will be noted that though only one definite pair of segments appear to be fused in the first three cases, there is in each instance an irregular mass of tissue between the separated edges of the fused pair of segments. Whether these masses are modified segments has not been determined.

In all four instances the nerve cords are formed on the side opposite the line of fusion. These cords are joined to the bilobed brains in a manner very similar to that found in No. 95 (Fig. 3). It is to be noted that the union of these individuals

is the same in kind and extent as in the group already described except for the digestive tract. In every one of the four there is distinguishable a separate alimentary tract for each member beginning with the mouths. These structures lie very near the nerve cord and are bounded on the inner side by a mass of muscles similar to those which usually lie on either side of the elongated fused pharynx.

The fixation of the blood vessels was such that they could not be studied.

The members of these groups to which belong half the specimens studied, have undoubtedly been fused along the dorsal side. The relative positions of setæ, nephridæ, and nerve cord are positive proof thereof. Vedjovsky gives a number of figures, very similar to Figs. 3 and 4, in his paper but he explains the union as being along the ventral side. It is impossible to disprove this conclusion for the two members were joined along their entire lengths. In the cases described in this paper, the union was never through more than five segments If, then, the coalescence had been other than dorsal, it is very probable that the nerve cord together with the setæ and excretory organs would have swung around to their natural position in the separated portions of the two individuals. This, however, is not the case. Therefore, the conclusion must be drawn that at least in these instances the union is no other than dorsal.

It is very probable that the one case described by Vedjovsky is also one of dorsal instead of ventral union, and that the cerebral ganglia are composed of half from each member instead of this being the case for the ventral cords, as that author supposes. If those individuals in which the digestive tract was separate had not been found it would not be possible to make this statement for it might be argued that each side of the pharynx was formed from the alimentary tract of one member. But since those cases have been found it can be easily seen that a joining of these structures would have resulted in forming a greatly elongated organ similar to that shown in Vedjovsky's figures. Furthermore, if the union in these forms had been ventral, the pharynges would certainly not have been found in the positions in which they lie, but would have been much nearer together in the central portion and side by side instead of end to end.

2. Those cases in which the union is latero-dorsal. Thus far only one example has been found of this group, individual No. 70 shown in Fig. 10, Plate II. Figs. 11 and 12, Plate II., show sections through the joined region which in this case extends through three segments. The first is very much more anterior than the second as is indicated by the presence of the cerebral ganglia (*bg*) of which there is in this monster only one set. The commissures extending from it can be seen in the same figure in which is also present the beginning of one of the nerve cords (*nc*). A study of the next few sections shows the connection of the brain to the two cords and also a connecting band between these two structures. In this manner the single greatly elongated alimentary tract, which in this case is the result of a union, is completely surrounded by nerve tissue. Fig. 12 gives a section through a more posterior region showing the relative positions of the ventral cords.

In no part of the joined portion can there be found less than eight pairs of setæ indicating that the union does not extend beyond the sides of the individuals.

The nephridæ also are in their normal number and relationship to the ventral cord.

3. This is the group in which the union is end to end and in which the cerebral ganglia are to be found on the opposite side of the digestive tract from the ventral cord. It can be seen from an inspection of Figs. 13, 14 and 15, external drawings of Nos. 90, 93 and 2, that the extent of union is limited to an unusually small area. Sections show that the union is across the dorsal side, for the ventral cords lie on the sides opposite that area, while the cerebral ganglia lie on the same side.

The digestive tracts have a common origin in each of the three monsters arising from a single mouth opening from which the pharynx passes into each individual. There is no noticeable difference in the structure of any of the fused organs of these forms as compared with those of No. 95 except in the nervous system.

A study of No. 90 will show a condition of that system somewhat different from any so far described. The ventral cord of each individual lies on the side opposite the union while the

brains lie on the same side and are connected to the cords by means of commissures, a condition very similar to that found in normal single individuals. The brains however in this instance are joined to each other. In one of the individuals the commissural connection between brain and cord can be seen beyond the point of separation, but in the other member the two ganglia of the brain seem to be separated and joined separately to the cerebral ganglia of the first member.

In No. 93, shown in Fig. 14, there is present only one set of cerebral ganglia greatly elongated. It lies on that side of the pharynx opposite the cords and is joined to that structure in one member by two commissures, to that in the other by only one.

A study of sections through No. 2 reveals a condition similar to that in No. 90. There are two distinct sets of cerebral ganglia, one joined to each nerve cord as in normal individuals and a connection between the two brains much as between the two cords in No. 70.

4. That group of double monsters in which the union between the two individuals has been side to side with both mouth openings on the same side, illustrated by Nos. 67 and 1.

In No. 1 the union is through one segment only as indicated in Fig. 16, Plate III. There is a single mouth opening lined with the columnar epithelial cells, from which the digestive tract of each individual passes inward. There can be seen on one side of the pharynx in a transverse section a single set of cerebral ganglia which is joined to the ventral cords by commissural strands extending over the pharynx. There are no such structures to be found on the under side of the alimentary tract. On the opposite side of the section there can be traced a connection between the cords themselves. Fig. 17, Plate III., is a section through the united portion of No. 1 showing the single pharynx, cerebral and ventral ganglia and a portion of the strand of nerve tissue connecting the two cords.

A study of sections through No. 67, shown in Fig. 18, Plate III., will show that the two mouth openings have been joined into one, and that the ventral cords lie on one side of the common pharynx and the cerebral ganglia on the other. There is only one set of brain ganglia to be found in this monster, but,

unlike all other cases, there are three distinct commissural strands joining it to the nerve cords, one extending over the top of the pharynx, the other two below the point of union of the two digestive tracts forming a triangle with each other. Fig. 19, Plate III., is a cross section of these last mentioned strands and their connection to the two cords. As in No. 70 there is in this monster also a limited connection between the two cords.

5. Cases in which the two individuals are extremely unequal in size, *e. g.*, bud formation. Thus far there have been found two such cases, a section of one being shown in Fig. 20, Plate III.

III. Discussion.

It will be remembered that reference was made to the difference in view concerning the origin of double monsters in an earlier part of this paper. Vedjovsky maintains that the formation of two embryos from one egg is abnormal, whereas Kleinenberg maintains the opposite. A difference of this kind may be explained on the basis of the difference in the forms worked on by these two investigators. This paper is a study of a form very similar, in its mode of development, to that described by Kleinenberg. It has been found that it is quite common, not only for monsters to appear in this group, but also for two individuals to emerge from one capsule. Out of the 184 cocoons opened 57 contained only one individual, 101 two, and 25 eggs in various cleavage stages and 1 four embryos. Thirty-five of the 101 cases were in the form of monsters. Six cases have been noted in which two or more eggs were present but in four of these only one was undergoing development while the others were in various stages of degeneration. One egg was found which had just reached a stage in development sufficiently far advanced to show the division into two hemispheres and the connecting band of larger cells very similar to Kleinenberg's Fig. 6. Three others were found which had begun to elongate in a manner very similar to the above.

It would certainly be safe to conclude that it is a common occurrence for one egg of this species to give rise to two individuals. If this were not the case it would seem rather unusual to find so large a number of cases in which two and only two

individuals emerge from one capsule when the number of eggs found in one capsule varies ordinarily from three to eight. The elongation of those eggs sufficiently far advanced in development to show this phenomenon is still further indication of this same thing.

Kleinenberg attributes the cause of this double embryo formation to the entrance of two sperm into a single egg setting up two points of activity. In the light of more modern discoveries this explanation no longer holds good. Vedjovsky suggests the possibility of temperature and moisture changes, and exposure to air bearing their influence on the egg and causing its abnormal development. Until further studies are made along this line it will be impossible to state any definite causes of monster formation.

BIBLIOGRAPHY.

Kleinenberg, N.

'78 Sullo Sviluppo del Lumbricus trapezoides.

Vejdovsky, Fr.

'88–'92 Ueber Zwillingsbildungen der Lumbriciden. Entwicklungsgeschichtliche Untersuchung.

Explanation of Plate I.

Helodrilus caliginosus trapezoides.

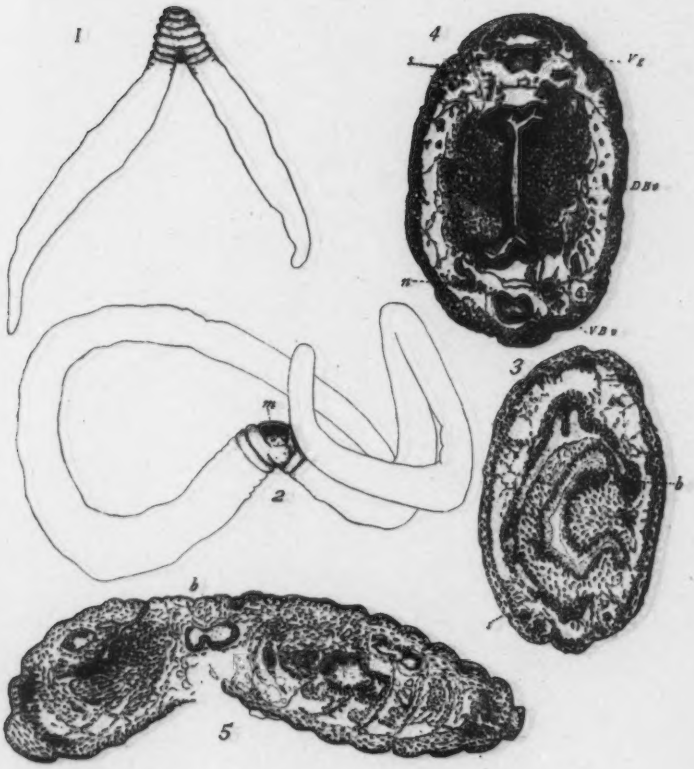
1. No. 95, showing union through five segments.

2. No. 171, showing union through one segment.

3. Section through the anterior portion of No. 95, showing the pharynx fused and the extension of the nerve tissue almost entirely around the pharynx. *b*, cerebral ganglia; *s*, setæ.

4. A section through a more posterior region of the same monster. *Vg*, ventral ganglia; *DBv*, dorsal blood vessel; *VBv*, ventral blood vessel; *n*, nephridium; *s*, seta.

5. A longitudinal section through No. 171, showing a portion of the cerebral ganglia *b*, and also parts of the ventral cords.



ROXIE A. WEBER.

Explanation of Plate II.

Helodrilus caliginosus trapezoides.

6. No. 91.
7. No. 92.
8. No. 172.

} Fused through one segment only with irregular masses between the separate edges of the united segment.

9. No. 173, united through four segments.

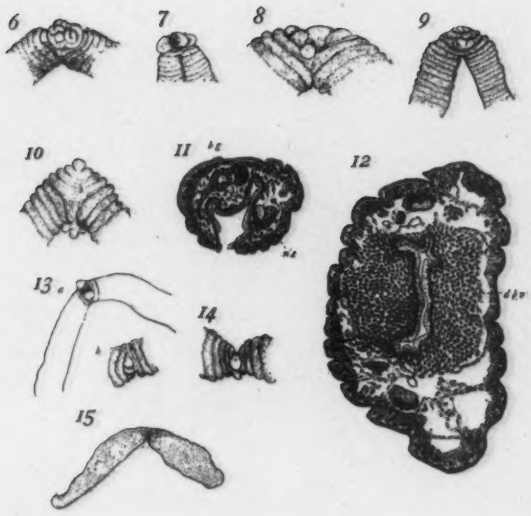
10. No. 70, united through four segments.

11. A section through the anterior portion of No. 70, showing a single set of cerebral ganglia (*bg*) and one of the ventral ganglia (*nc*).

12. A more posterior section of the same monster showing two ventral ganglia.

13*a* and *b*. No. 90.
14. No. 93.
15. No. 2.

} All show very limited connection.



ROXIE A. WEBER.

Explanation of Plate III.

Helodrilus caliginosus trapezoides.

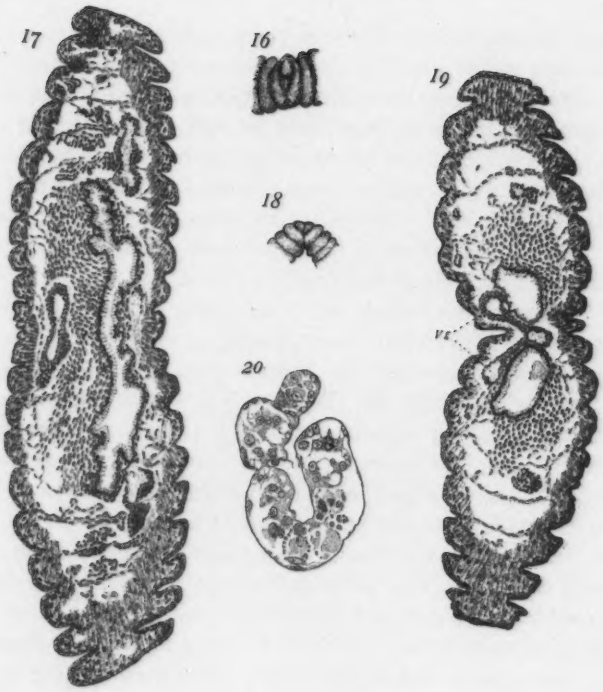
16. No. 1 viewed from the side of the mouth opening.

17. A section through No. 1 horizontal to the mouth opening, showing the cerebral ganglia on one side of the pharynx and the cord tissue on the opposite side.

18. No. 67, united through two segments.

19. A transverse section through the posterior part of the united portion showing the cerebral ganglia joined to the ventral ganglia (*vg*) by commissures.

20. A section through a gastrula stage showing bud formation.



ROXIE A. WEBER.

IODINE AS A PARTHENOGENETIC AGENT.

ALVALYN E. WOODWARD AND FLORENCE S. HAGUE.

In the course of some experiments with *Arbacia* eggs at Woods Hole last summer, it was noted that, if a little iodine was added to the eggs in sea-water, regular fertilization membranes appeared on a large number. This led to an attempt to find the optimum conditions for the use of this reagent in parthenogenesis.

The stock solution used was made by dissolving iodine crystals in sea-water to the point of saturation. This stock solution was diluted with sea-water as shown in the tables.

Some of the females were stimulated to shed their eggs into watch glasses. In other cases, the ovaries were removed and shaken in sea-water to loosen them. Whichever method was followed, the eggs were washed and allowed to settle. The supernatant water was poured off, the eggs evenly mixed in fresh sea-water, and 2 c.c. of the mixture put into each of a series of watch glasses. To each of these an equal volume of iodine solution was added, for a given length of time. Some of the eggs were then put into finger-bowls containing about 50 c.c. of sea-water. Others were treated for 20 minutes with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2.5 N NaCl). At the end of that time, the solution was drawn off and the eggs transferred to finger bowls of sea-water. With each lot of eggs, three controls were made, one fertilized with sperm, one uninseminated, and one uninseminated but treated with hypertonic sea-water.

Table I. shows the percentage of membranes and cleavages produced by varying the strength of the solution and the length of exposure. To obtain these averages, over two hundred eggs were counted in each case.

It will be noted that there is great variability in the results with different batches of eggs. This may be explainable from the fact that the experiments were carried on during the last two weeks of August and the first week of September, a period

TABLE I.

PERCENTAGE OF CLEAVAGES AND MEMBRANES OBTAINED BY TREATING ARBACIA EGGS WITH IODINE, OR IODINE FOLLOWED BY HYPERTONIC SEA-WATER.

Time, Min.	Sat. Iodine Solution.		1/8 Sat. Iodine Solution.		1/4 Sat. Iodine Solution.		1/8 Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Sea-water.	Not Fertilized. In Hypertonic.	
2.5			14	24											69	3	5	Shed
			14	22											64	2	2	Shaken
	2	7	17	16											68	4	2	Shaken
					26	27	16	16							41	1	4	
					16	32	11	39							69	3	11	
									12	13	12	14			85	7	9	
									5	8	4	12			83	7	9	
5					16	12	14	39	6	16	3	3			44	2	2	Shed
					18	21	30	22	17	17	5	15			32	1	2	Shed
					3	15	20	22	20	23	9	10			83	1	4	Shaken
			1	1	21	2	20	29	14	23					82	5	6	Both
							12	32							3	1	1	Shed
							19	20							94	5	6	Shaken
							16	27							94	5	6	Shaken
									8	21					66	3	5	Both
			20	17											69	3	5	Shed
			19	7											64	2	2	Shaken
	1	1	18	9											68	4	2	Shaken
					49	49	11	5							41	1	4	
					23	37	26	43							69	3	11	
									10	13	9	4			85	7	9	
									2	13	5	10			83	7	9	

TABLE I.—*Continued.*

Time, Min.	Sat. Iodine Solution.	+ Hypertonic.	½ Sat. Iodine Solution.	+ Hypertonic.	¼ Sat. Iodine Solution.	+ Hypertonic.	⅛ Sat. Iodine Solution.	+ Hypertonic.	1/16 Sat. Iodine Solution.	+ Hypertonic.	1/32 Sat. Iodine Solution.	+ Hypertonic.	1/64 Sat. Iodine Solution.	+ Hypertonic.	Controls. Fertilized.	Not Fertilized. In Seawater.	Not Fertilized. In Hypertonic.	Remarks.
7.5			5	21	29	20	16	13	1	2					13	0	1	Shed
			35	37	28	28	41	31	8	18					70	4	10	Shaken
							21	47							3	1	1	Shed
							30	22							94	5	6	Shaken
							22	27							94	5	6	Shaken
									9	26					66	3	5	Shed
			15	13											69	3	5	Shed
			11	10											64	2	2	Shaken
	7	4	23	16											68	4	2	Shaken
					34	24	24	27							41	1	4	
					25	36	28	27							69	3	11	
10			13	7	23	20	21	19	11	16					88	1	5	Shed
			0	15	30	26	25	19	8	21					51	0	3	Shed
			3	9	37	25	13	33	7	17					88	1	11	Shaken
			3	15	22	16	14	18	11	6					13	0	1	Shed
			28	23	19	23	11	21	23	32					70	4	10	Shaken
			1	1	1	8	26	31	28	28					82	5	6	Both
							45	56							3	1	1	Shed
							19	24							94	5	6	Shaken
							26	37							94	5	6	Shaken
									16	24					66	3	5	Both
			8	5											69	3	5	Shed
			7	14											64	2	2	Shaken
	3	3	11	17											68	4	2	Shaken

TABLE I.—*Continued.*

Time, Min	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Seawater.	Not Fertilized. In Hypertonic.	
10					32	44	18	14							41	1	4	
					26	39	18	44							69	3	11	
									15	12	4	15			85	7	9	
									6	17	4	10			83	7	9	
12.5							8	28							3	1	1	Shed
							13	18							94	5	6	Shaken
							23	24							94	5	6	Shaken
									16	26					66	3	5	Both
			12	6											69	3	5	Shed
			7	19											64	2	2	Shaken
	11	3	8	16											68	4	2	Shaken
					18	20	19	46							69	3	11	
15							15	33							3	1	1	Shed
							16	22							94	5	6	Shaken
							17	41							94	5	6	Shaken
									21	35					66	3	5	Both
					24	42	29	29							69	3	11	
									9	11	10	12			85	7	9	
					21	27	7	13					2	5	92	1	2	Shaken
20									15	17	14	15			85	7	9	
					21	24	9	5					2	8	92	1	2	Shaken
25									15	20	8	16			85	7	9	

near the close of the breeding season, when the eggs vary greatly in their fertilizing power and their sensitiveness to reagents. It is probable that the strength found best for this season will prove too great for the more sensitive eggs in the height of the season—July, for instance.

Table II. is a summary of Table I., giving the average number

TABLE II.

SUMMARY OF TABLE I.

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.	
		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.
2.5	2	7	15	21	21	30	14	28	9	11	8	13		
5	1	1	15	9	22	23	19	27	11	18	6	8		
7.5	7	4	18	19	29	27	26	28	6	15				
10	3	3	6	12	24	25	20	26	14	19	4	13		
12.5	11	3	9	14	18	20	16	29	16	26				
15					23	35	17	28	15	23	10	12	2	5
20					21	24	9	5	15	17	14	15	2	8
25									15	20	8	16		

of cleavages plus membranes obtained by each method of treatment. A number in italics is the result of one experiment, rather than the average of several. There is a remarkable uniformity in the percentages obtained by treating the eggs for varying periods of time. For instance, whether exposed for two and one half or fifteen minutes, or for some intermediate period, to one eighth saturated iodine, the eggs gave percentages between 26 and 28. The indication is that the iodine enters the egg immediately and affects it immediately to its full extent. One would expect this result if it acted by combining chemically with something in the egg. While length of exposure has no effect, varying the strength of iodine used causes great variation in the result. This indicates a secondary, non-initiatory or injurious effect due to the excess. The optimum results usually followed treatment of 2 c.c. eggs in sea-water with 2 c.c. of one fourth or one eighth saturated iodine solution. While hypertonic after treatment was nearly always beneficial, in most cases the effect was so slight as to be negligible.

It may be recalled that membrane formation and subsequent

cytolysis were obtained by Loeb,[1] McClendon,[2] and Glaser,[3] in *Arbacia* eggs, by placing them in sea-water diluted with distilled, or in pure distilled water. In the latter case, the eggs became "ghosts" of their former selves, since the pigment dissolved out and increased osmotic pressure caused the egg to absorb water and swell enormously. If an excess of iodine is added to normal eggs, they also become pale, but due to a precipitation of the pigment, which gathers as a small dark mass near one side of the egg. There is seldom indication of swelling or ghost formation.

The membranes obtained by the iodine method were identical with the true fertilization membranes which appear when the sperm enters the egg. They do not resemble the membranes formed by treatment with butyric acid and hypertonic sea-water, which Loeb describes as very difficult to see, excepting by the trained observer. This normal appearance of the membranes was noted by others accustomed to distinguishing types of membrane in *Arbacia*. The significance of the nature of this membrane will be discussed in a later paper.

Reference to Table I. brings out a result that was wholly unexpected. It is commonly supposed that eggs shed by the animal are in better condition and more fertile than those taken from the ovary. The results with one female, whose shed eggs produced only 3 per cent. cleavages, while those shaken from the same ovaries gave 94 per cent., were so striking that the averages were computed. It was found that the shed eggs averaged only 43 per cent. as against 80 per cent. cleavages from the eggs shaken from the ovaries. It is planned to test this during the height of the breeding season, so as to discover whether this is a normal occurrence or whether the "shed" eggs are more likely to be "over-ripe" than the others, late in the season.

[1] Loeb, Jacques, "Die chemische Entwicklungserregung, etc.," Julius Springer, Berlin, 1909.

[2] McClendon, J. F., "On Artificial Parthenogenesis of the Sea-urchin Egg," *Science*, 1909, Vol. 30, pp. 454–455.

[3] Glaser, O. C., "On Inducing Development in the Sea-urchin (*Arbacia punctulata*), Together with Considerations on the Initiatory Effect of Fertilization," *Science*, 1913, Vol. 38, pp. 446–450.